Identifying Tumor Suppressor Genes in Human Colorectal Cancer

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APPROXIMATELY A DECADE AGO, THE MOLECULAR GENETIC era of human cancer was ushered in with the discovery of "dominantly acting" activated cellular oncogenes (*1*). The first activated oncogenes were isolated by transfection of DNA from human cancer cells into mouse NIH 3T3 cells, a process that resulted in neoplastic transformation. The activated oncogenes were quickly found to be homologs of retroviral transforming genes (*2*). This finding, which was predicted on the basis of the seminal studies that showed that the avian retroviral src oncogene had evolved from the capture of a cellular protooncogene (*3*), led to the further identification of numerous candidate cellular oncogenes.

The discovery that activated oncogenes could be found in 10 to 30 percent of human cancers led to theories that activation of single or multiple cooperating cellular oncogenes was in itself sufficient to create a cancerous cell. These theories were all the more attractive when it was found that the expanding list of oncogene functions included growth factors, growth factor receptors, signal transducers, protein kinases, and transcriptional activators—all of which, when behaving aberrantly, might lead to uncontrolled cell proliferation.

A tacit assumption in many interpretations of these studies was the dominant nature of activated oncogenes. However, earlier studies with somatic cell hybrids had clearly shown that when malignant cells were fused with normal cells, the resulting hybrid cells were nontumorigenic (*4*). This phenomenon of tumor suppression indicated that a gene (or genes) from a normal cell might replace a defective function in the cancer cell and render it responsive to normal regulators of cell growth.

The notion of loss of genetic function being a critical event in the genesis of cancer received further support when it was shown by a combination of cytogenetic and molecular studies of restriction fragment length polymorphisms (RFLP's) that specific chromosomal deletions are often associated with certain human malignancies (*5*). The combination of these studies led to the hypothesis that a class of genetic elements, termed tumor suppressor genes, exist which must be inactivated in some fashion—for example, by deletion, point mutation, or methylation—before a cell can become malignant (*6*).

Final proof of this hypothesis was obtained when the tumor suppressor retinoblastoma gene (Rb-1) was cloned a little over 2 years ago (*7*). Retinoblastoma cells lack a functional Rb protein. When the wild-type Rb-1 gene is introduced into these cancer cells, their tumor-forming property is suppressed (*8*). Recent data suggest that the retinoblastoma protein plays a critical role in control of the cell cycle (*9*).

In certain malignancies, such as retinoblastoma, loss of function of only a single tumor suppressor gene is implicated. However, in other cancers, RFLP studies have indicated that loss of multiple tumor suppressor genes may be necessary for progression to the fully malignant condition. Examples of such malignancies include small cell lung cancer, breast cancer, and colorectal cancer (*10*).

Colorectal cancer is rapidly becoming the most extensively characterized human malignancy in terms of the molecular genetic alterations associated with its progression. This is in part because, unlike many other malignant conditions, tissues that represent the progression from normal mucosa through hyperplasia, benign adenoma to carcinoma in situ and finally metastasis are readily identifiable (Fig. 1). Vogelstein *et al.*, as well as other investigators, have taken advantage of this by obtaining tissues that represent the various stages of progression to colorectal carcinoma from the same patient and screening the DNA from these samples for activated oncogenes and loss of RFLP heterozygosities that would be indicative of loss of tumor suppressor gene function. The following changes were reproducibly observed in sporadic colorectal carcinoma DNAs: activation of the Ki-*ras* oncogene in approximately 40 to 50 percent of cases and loss of genetic information from chromosomes 5q21-q22 (about 35 percent of cases), 17p12-p13 (more than 70 percent of cases) and 18q21-qter (more than 70 percent of cases) (*11*). Thus a case could be made for the role of both activation of an oncogene (albeit early in progression) and inactivation of multiple tumor suppressor genes in this particular malignancy. The involvement of 5q21-q22 in sporadic colorectal cancer is intriguing since this region has been identified by linkage analysis as the locus of the autosomal dominant familial adenomatous polyposis (FAP) gene which predisposes to a high incidence of colorectal cancer (*12*).

The race was now on to clone these candidate tumor suppressor genes. The first to come forth was the gene on chromosome 17p. Vogelstein and his colleagues noted that a previously cloned p53 gene mapped to this region and checked its status in a number of colonic cancers. Sure enough, each tumor showed evidence of partial or complete deletion of one allele of the p53 gene (*13*). They then sequenced the other allele (genomic or cDNA) and found in each case that at least one point mutation could be identified. Thus, the p53 gene fitted the criteria for a tumor suppressor gene, that is, inactivation by a combination of allele loss and point mutation. The disturbing feature of this scenario, however, was that p53 had already been identified as an oncogene! It had originally been discovered by Crawford and colleagues as a protein located in the nucleus of both normal and transformed cells (*14*). Much higher expression was found in both rodent and human cancer cells compared to normal cells and was probably related to the considerably shorter half-life of the p53 protein in normal as compared to transformed cells (on the order of 15 minutes versus several hours, respectively). The p53 gene was then cloned and found to act as an oncogene, cooperating with *ras* to transform primary rodent fibroblasts (*15*). A resolution of this paradox was reported almost coincidentally with Vogelstein's findings. It was found that the original mouse p53 cDNA that had been cloned contained a point mutation. Studies with the wild-type p53 gene now indicate that it inhibits transformation of primary rodent cells by *myc* and *ras*, a function more in keeping with a tumor suppressor gene (*16*). A final feature of these studies is that Levine and colleagues have evidence that the presence of only one mutant p53 allele is enough to convey

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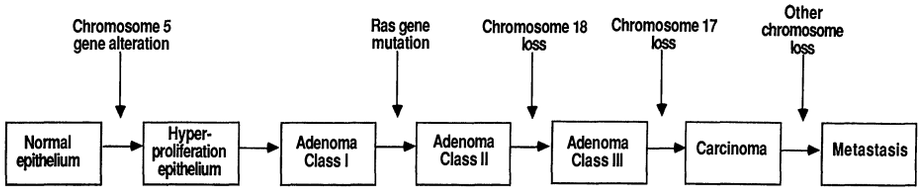

Fig. 1. A model for colorectal tumorigenesis. Tumorigenesis proceeds through a series of genetic alterations, including *ras* gene activation and loss of putative tumor suppressor genes on chromosomes 5, 17, and 18. [Adapted from a figure by B. Vogelstein *et al.* (personal communication) with permission]

a transforming effect on cultured mouse cells, a finding interpreted as a dominant negative mutation. The p53 protein is a DNA binding protein that functions as a homodimer. It is thought that complexing of one mutant subunit with a wild-type subunit is sufficient to subvert normal function. This paradigm has yet to be found in human cells.

The most recent progress has now been the cloning of the candidate tumor suppressor gene on chromosome 18q21-qter by Vogelstein and colleagues (*Science*, this issue, page 49). In a technical tour de force, Fearon *et al.* used probes that mapped to the chromosome 18q21-qter region and noted with one probe a carcinoma that had a homozygous deletion in this region. In other cases they also noted allelic loss and one interesting situation where a "gain" of heterozygosity was seen. The compilation of data was sufficient for them to attempt cloning of expressed sequences in the region. After many futile attempts with candidate exon probes, they attempted to improve the sensitivity of the expression assays using the polymerase chain reaction in a novel exon-connection strategy. This finally proved successful and allowed for the isolation of a partial cDNA clone mapping to 18q21. The gene is expressed in many tissues, including normal colonic mucosa, with the highest expression found in brain tissue. However, there is absent or drastically reduced expression in colorectal carcinomas. This absent or reduced expression is consistent with a tumor suppressor function.

The gene is very large with an mRNA transcript size of 10 to 12 kb. Somatic mutations within the gene have been observed in a number of colorectal cancers; these include a homozygous deletion at the 5′ end of the gene, a point mutation within one of the introns, and ten examples of DNA insertions within a fragment immediately downstream of one of the exons. The significance of these alterations is unresolved, since a full-length cDNA or genomic clone is not as yet available.

Most intriguingly, this gene termed DCC (deleted in colorectal carcinomas) shows significant homology to neural cell adhesion molecules (CAM's) and other related cell surface glycoproteins. The DCC gene contains four immunoglobulin-like domains of the C2 class and a fibronectin type III–related domain similar to the domains present in N-CAM, LI, and other members of this family of CAM.

The fact that DCC is related to genes involved in cell-surface interactions is clearly provocative. There is an abundance of evidence that disruption of cell adhesion and cell communication are critical events in neoplastic transformation. Disruption of normal cell-cell contacts is often noted in the process of metastasis and intercellular adhesion mediated by CAM's directly influences differentiation (*17*), a process often disrupted in malignancy.

In view of the accumulating information on tumor suppressor genes it is becoming clear that, as with activated oncogenes, there are many such genes with diverse functions. It has already been demonstrated that alterations of both the Rb-1 and the p53 genes are found in several human cancers. This is suggestive of potentially common pathways of negative regulation of cell growth that must be disrupted to allow for malignant growth of several different tumors. Up until now alterations of the DCC gene have been found only in colorectal carcinomas. However, given its high expression in brain and other tissues, it is likely that tumors that originate in these tissues will be closely examined for defects in expression of this gene.

The molecular identification of several candidate oncogene and tumor suppressor genes in colorectal carcinoma is exciting and we eagerly await the cloning of the FAP gene on 5q. The caveat that must be entered here is that we know nothing as yet about the functional significance of any of these genetic alterations. Studies with the p53 gene are in progress and hopefully a full-length cDNA of DCC will soon be available for study. Whether correction of any one of the defects in a colorectal carcinoma that carries multiple alterations is sufficient to reverse malignancy or whether complete restitution is necessary is still unknown.

One must also hope that future molecular genetic and functional studies will identify promising avenues for improved diagnosis and treatment of this disease.

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